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Protocol Review

Deciphering Hematopoietic Stem Cells in Their Niches: A Critical Appraisal of Genetic Models, Lineage Tracing, and Imaging Strategies

Chacko Joseph,[1,2] Julie M. Quach,[1] Carl R. Walkley,[1,2] Steven W. Lane,[3] Cristina Lo Celso,[4] and Louise E. Purton[1,2,*]
[1]Stem Cell Regulation Unit, St. Vincent's Institute of Medical Research, 9 Princes Street, Fitzroy, VIC, 3065, Australia
[2]Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Fitzroy, VIC, 3065, Australia
[3]QIMR Berghofer Medical Research Institute, University of Queensland, Brisbane, QLD, 4029, Australia
[4]Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK
*Correspondence: lpurton@svi.edu.au
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In recent years, technical developments in mouse genetics and imaging equipment have substantially advanced our understanding of hematopoietic stem cells (HSCs) and their niche. The availability of numerous Cre strains for targeting HSCs and microenvironmental cells provides extensive flexibility in experimental design, but it can also pose significant challenges due to strain-specific differences in cell specificity. Here we outline various genetic approaches for isolating, detecting, and ablating HSCs and niche components and provide a guide for advantages and caveats to consider. We also discuss opportunities and limitations presented by imaging technologies that allow investigation of HSC behavior in situ.

Introduction

Hematopoietic stem cells (HSCs) in the adult human and mouse predominantly reside in the bone marrow (BM), where blood cell development occurs. The BM also contains nonhematopoietic components such as bone-forming cells (including mesenchymal stem cells [MSCs] and osteoblast [Ob]-lineage cells), adipocytes, vasculature, innervation from the sympathetic nervous system, and other stromal cells such as adventitial reticular cells, pericytes, and fibroblasts (Figure 1). It has long been recognized that nonhematopoietic BM-derived stromal cells are capable of supporting long-term hematopoiesis in vivo and in vitro (Dexter et al., 1973, 1977; Schofield, 1978; Trentin, 1971; Wolf and Trentin, 1968). However, until recently little was known about the anatomical location of HSCs within the BM microenvironment (HSC niche), and the identities of the HSC-supporting stromal cells were not well defined, limiting our understanding of how HSCs are regulated.

Since the early 2000s there has been a significant improvement in our understanding of the cell types that contribute to the regulation of hematopoiesis and in particular HSCs (for recent examples see Krause et al., 2013; Nakamura-Ishizu and Suda, 2013). These advances have largely come from the development of a broad range of genetically modified murine models (both transgenic and targeted alleles) coupled with concurrent innovation in imaging technologies. Here we review some of the most commonly used transgenic strains of mice for detection/deletion studies in HSCs and microenvironment cell types and highlight the advantages and limitations of these strains where applicable. Our aim is to provide a useful guide for designing and interpreting studies using the various strains that are available. This review may help to explain why studies investigating the effects of a given gene in a certain cell type (e.g. HSC) can have different outcomes when different Cre transgenic strains are used to target the cell. We also discuss emerging imaging strategies for interrogating HSC behavior in situ and explain the benefits and caveats of different approaches.

Genetically Modified Mouse Models Currently Used to Study HSCs and Their Interactions with HSC Niches

Genetically modified mice have been widely applied to study either HSCs or specific HSC niche cell types within the BM. The ability to control the time and location of somatic mutations in adult mice has allowed us to answer specific questions regarding the roles of different cell populations in the regulation of HSC homeostasis. These tools have been applied to determine the functional consequence of deleting single genes in specific cell types (Table 1). Additionally the use of reporter systems to illuminate a given cell lineage has enabled lineage-tracking methods that allow prospective isolation and/or identification of a cell of interest. Note that there still remain limitations to the interpretation of studies using these animal models. For example, the inability to detect a phenotype in a cell-specific conditional knockout mouse may not necessarily mean it has no importance in the HSC niche because functional redundancy can occur and/or other important factors may be produced by that cell type to regulate HSCs.

Cre-Driven Transgenic Mice Commonly Used to Identify HSCs

Myxovirus resistance-1 (Mx1-Cre). Mx1 is a vital part of the viral defense mechanism and its expression can be highly induced in response to interferon. Cre recombinase under the control of the Mx1 promoter can be activated by mimicking viral infection by the administration of synthetic double stranded RNA [poly(I):poly(C)], an *interferon-α/β* (IFN) activator (Kühn et al., 1995). There is a low detectable rate of Cre induction generated by endogenous interferon (Kühn et al., 1995), and as a result phenotypes have been observed in some strains in the absence of pIpC treatment (Hartner et al., 2009; Isakoff et al., 2005). Furthermore, IFN itself influences HSC biology at levels much lower

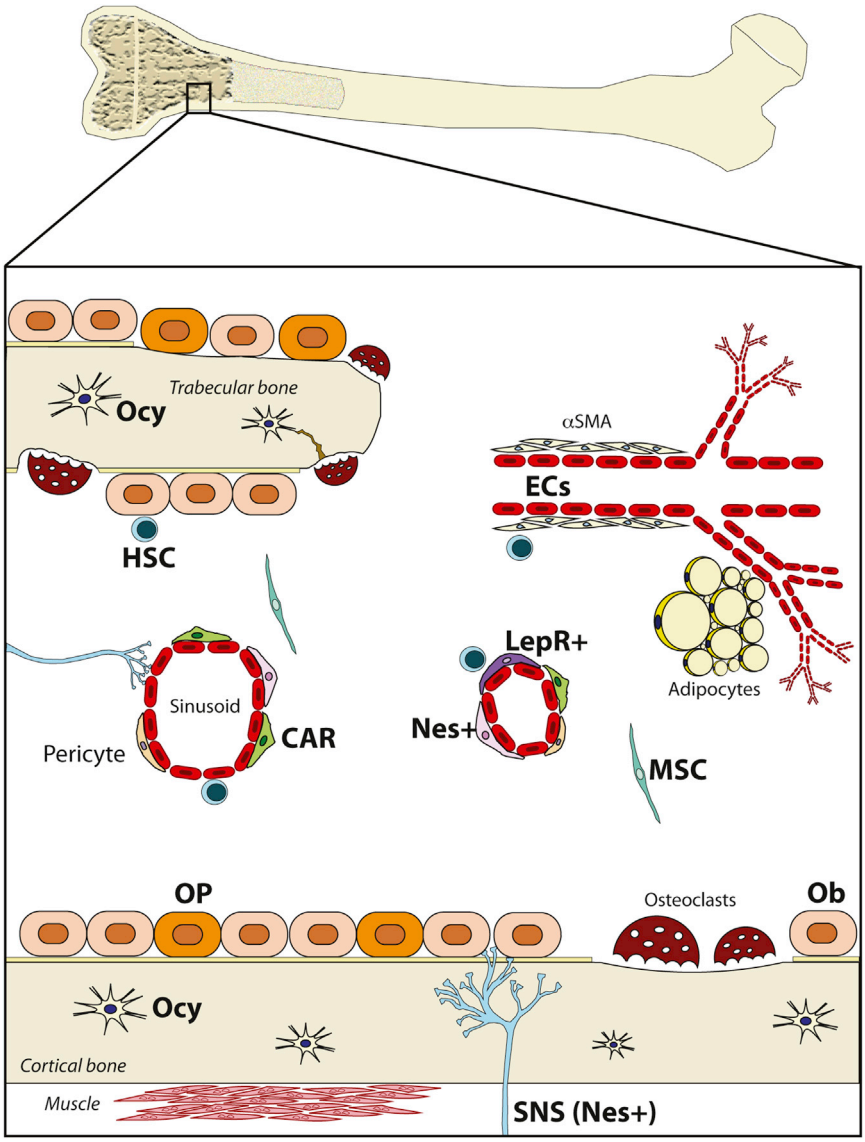

Figure 1. The Complexity of HSC Niches
The major nonhematopoietic cell types in the BM microenvironment that have roles in regulating HSCs are shown. Genetically modified mouse models that can target the cell types highlighted in bold are discussed in this Protocol Review. Other cell types (lightface) may also have roles in regulating HSCs, but have not yet been explored in detail using transgenic mouse strains, and hence are not discussed here. Some cell types (e.g. LepR+ and CAR cells) may be the same cell but this has not yet been confirmed, so they are represented individually. Italicized words refer to tissue structures within the BM. MSC, mesenchymal stem cell; OP, osteoprogenitor; Ob, osteoblast; Ocy, osteocyte; CAR, CXCL12-abundant reticular cell; αSMA, α-smooth muscle cell actin-expressing cells; Nes+, nestin-positive cell; LepR+, leptin receptor positive cell; EC, endothelial cell; SNS, sympathetic nervous system.

than that generated by pIpC used for *Mx1-Cre* activation, which may complicate the phenotype observed (Essers et al., 2009; Hall et al., 2003; Hartner et al., 2009; Zhao et al., 2007).

Cell specificity. Mx1-Cre has been shown to be active in several tissues including liver, kidney, and heart (Kühn et al., 1995). *Mx1-Cre*-targeted cells also include immature MSCs in BM that were capable of forming Ob, chondrocyte, and adipocyte lineages in vitro (Park et al., 2012). In vivo, however, *Mx1-Cre* YFP+ cells predominantly formed Ob-lineage cells and rarely adipocytes, with no *Mx1-Cre* YFP+ chondrocytes observed (Park et al., 2012). Furthermore, Park et al. demonstrated that more than 50% of nestin-positive cells in the BM were also targeted by *Mx1-Cre*, and that *Mx1-Cre*-GFP+ perivascular cells were visualized close to the endosteum (Park et al., 2012). *Mx1-Cre* did not target BM endothelial cells (ECs), skeletal muscle cells, or fibroblasts in the BM of the mice.

Vav-Cre. Vav is a guanine nucleotide exchange factor regulated by the Rho/Rac family of small G proteins (Lazer and Katzav, 2011). The constitutively active *Vav-Cre* mice have been commonly used to achieve gene deletion in the hematopoietic system. Two *Vav-Cre* strains have been generated, having only slightly different specificity (Croker et al., 2004; de Boer et al., 2003; Georgiades et al., 2002). In one strain (*Vav-Cre*), the mice were generated by the expression of Cre under the control of the murine *vav* gene regulatory elements (Croker et al., 2004; de Boer et al., 2003; Georgiades et al., 2002). In the other, the targeting vector (Ogilvy et al., 1999) was modified with the codon-improved Cre (iCre) cDNA (which is less susceptible to epigenetic silencing) (Shimshek et al., 2002) and used to generate transgenic *Vav-iCre* mice (Croker et al., 2004; de Boer et al., 2003; Georgiades et al., 2002).

Cell specificity. Both *Vav-Cre* strains have been shown to target hematopoietic cells and ECs, with Cre recombinase activity also reported either in the testes in the *Vav-iCre* mice (Croker et al., 2004; de Boer et al., 2003) or the ovaries in the *Vav-Cre* mice (Croker et al., 2004; de Boer et al., 2003; Georgiades et al., 2002). This has resulted in some offspring with germline deletion (A. Roberts, personal communication). Both strains should always be bred as heterozygous floxed mice with Cre+ males for the *Vav-Cre* matings and Cre+ females for the *Vav-iCre* breeders to assist in avoiding this issue.

Tie2-Cre. The Tie2 gene encodes a tyrosine kinase family receptor specific to angiopoietin. Tie2 is expressed by ECs and is important for angiogenesis (Maisonpierre et al., 1997; Schnürch and Risau, 1993). However, Tie2 is also expressed by hematopoietic cells and plays a critical role in HSC quiescence (Arai et al., 2004; Takakura et al., 1998). Two strains of transgenic mice expressing constitutively active Cre recombinase under the control of the Tie2 promoter were initially generated to achieve endothelial-cell-specific deletion in vivo

Table 1. A Summary of HSC-Cre Mice, Their Advantages, and Their Limitations

Mouse Strain	Inducible/ Repressible	Specific to HSCs?	Germline Deletion?	Recommended Mating Strategy[a]	Other Potential Issues Associated with this Strain
Mx1-Cre	pIpC (IFN) inducible	no: other cell types include BM MSCs, nestin+ cells, perivascular cells, and cells in other tissues	no	only one sex Cre+, no preference	low levels of IFN present normally in mice can induce Cre recombinase activity in some cells
Vav-Cre	no	no: also detects ECs and cells in the ovaries	yes	Cre+ male mated to Cre− female	none reported
Vav-iCre	no	no: also detects ECs and cells in the testes	yes	Cre+ female mated to Cre− male	none reported
*Tie2-Cre**	no	yes: endothelial cells	yes	Cre+ male mated to Cre− female	none reported
HSC-SCL-Cre-ER^T	tamoxifen-inducible	not when induced in adult mice; however, if given to embryos, also detects ECs	no	Only one sex Cre+, no preference	none reported

pIpC, poly(I):poly(C); IFN, interferon; MSCs, mesenchymal stem cells; ECs, endothelial cells. (*) indicates information relevant to all strains generated.
[a]It is very strongly recommended for all strains to use heterozygous breeders to generate age- and sex-matched littermate floxed/floxed (fl/fl) and wild-type (WT) controls. Breeders consisting of a Cre− and Cre+ pair are optimal for many strains because potential issues may arise if both mice in the breeding pair are Cre+. For example, homozygosity in knockin Cre alleles disrupts expression of the endogenous gene generating a knockout of that gene. Furthermore, it is highly recommended that groups of control mice that consisting of Cre− fl/fl and Cre+ WT genotypes should be investigated in studies determining the impact of gene deletion in a specific cell lineage to control for any impact that the floxed allele and the Cre transgene may have on the cells and the animal, such as Cre toxicity (Turan et al., 2011). Potential effects of haploinsufficiency can be investigated in the Cre+ floxed/+ mice. Germline deletion should be monitored in all strains that are susceptible to this off-target effect.

(Constien et al., 2001; Kisanuki et al., 2001). Consistent with their roles in angiogenesis and hematopoiesis, *Tie2-Cre* mice display Cre recombinase in both ECs and hematopoietic cells, especially HSCs (Constien et al., 2001; Tang et al., 2010). When crossed to EGFP or eYFP reporter mice, both *Tie2-Cre* strains were shown to have Cre expression in 80%–90% of the hematopoietic cells (Constien et al., 2001; Tang et al., 2010). No differences appear to exist between specificity of these strains; however, slight differences may arise based on where the transgenes have integrated into the DNA of the mice.

While BM transplantation (BMT) studies can be performed to test HSC potential, the deleted ECs may have altered the potential of the HSCs prior to transplant; hence, any phenotype observed may not be intrinsic to HSCs. Furthermore, it has been shown that Sca-1+ lineage-negative BM cells contain transplantable endothelial progenitor cells (Grant et al., 2002); thus, the chimeras obtained upon transplantation have the potential to contain some Tie2-Cre+ ECs in addition to HSCs. For these reasons it is strongly advised that studies using *Tie2-Cre* mice should investigate the potential contribution of both endothelial and hematopoietic cells to the phenotype observed.

At least two other *Tie2-Cre* strains have been generated (Koni et al., 2001; Theis et al., 2001), in addition to a Tie2-GFP strain using the same construct (Motoike et al., 2000). All have shown expression in ECs, but none of these have, to our knowledge, been extensively investigated to determine whether there is also expression in hematopoietic cells. However, given that they used the same original vector that the other two strains were slightly modified from (Schlaeger et al., 1997), they are highly likely to show activity in hematopoietic cells.

Recently, two groups used different *Tie2-Cre* strains to show that endothelial-derived CXCL12 is important in HSC maintenance (Ding and Morrison, 2013; Greenbaum et al., 2013), with the Morrison lab using the Koni et al. strain (Koni et al., 2001) and the Link lab using the Kisanuki et al. strain (Kisanuki et al., 2001). Endothelial-cell-derived SCF was also shown to be important in regulating HSCs by Ding et al. (2012) using the Koni et al. strain (Koni et al., 2001).

Cell specificity. Investigators using *Tie2-Cre* mice should be cautioned that progeny of *Tie2-Cre* mice can include offspring with germline Cre expression (de Lange et al., 2008; Koni et al., 2001). The Cre transgene has been shown to be transmitted through the female germline, which can be avoided by choosing Cre+ males to breed to Cre− females (de Lange et al., 2008; Koni et al., 2001).

HSC-SCL-Cre-ER^T. The stem cell leukemia (Scl) gene is known to play an indispensable role in both developmental hematopoiesis and angiogenesis (Robb et al., 1995; Visvader et al., 1998). Tissue-specific Scl expression is dictated by two distinct regulatory elements: the 5′ enhancer mainly driving the endothelial Scl expression and the 3′ enhancer mainly dedicated to hematopoietic Scl expression (Göttgens et al., 2002, 2004). Capitalizing on this regulatory mechanism, Göthert et al. developed inducible tissue-specific Cre-recombinase-expressing transgenic mice for hematopoietic cells (*HSC-SCL-Cre-ER^T*) (Göthert et al., 2005) and ECs (*endothelial-SCL-Cre-ER^T*) (Göthert et al., 2004). Tamoxifen-inducible Cre recombinase (Cre-ER^T) was developed by fusing a mutated estrogen receptor with the Cre recombinase enzyme (Feil et al., 1996).

Cell specificity. Continuous administration of tamoxifen for 2 weeks (via chow) in *HSC-SCL-Cre-ER^T* adult mice resulted in Cre recombinase expression in 90% of long-term HSCs (identified as lineage$^-$c-Kit$^+$Sca-1$^+$Flt3$^-$) (Göthert et al., 2005). When tamoxifen was administered prenatally (by feeding the mothers), it was revealed that a population of embryonic-derived HSCs persisted and repopulated hematopoiesis long-term in adult

Table 2. A Summary of Vascular/Perivascular Targeting Cre Mice, Their Advantages, and Their Limitations

	Mouse Strain	Inducible/ Repressible	Specific to the Cell of Interest?	Germline Deletion?	Recommended Mating Strategy	Other Potential Issues Associated with This Strain
Endothelial cell strains	*Tie2-Cre*	no	no: HSCs also detected	yes	Cre+ male mated to Cre− female	none reported
	VE-Cadherin-Cre	no	no: HSCs also detected	no	Only one sex Cre+, no reported preference	none reported
	VE-Cadherin-CreER^{T2}	tamoxifen-inducible	not when induced in adult mice; however, if given to embryos, also detects HSCs	no	Only one sex Cre+, no reported preference	postnatal induction of Cre recombinase activity does not target all ECs
	VE-Cadherin: tTA-Cre	tetracycline-inducible	has not been investigated but likely the same as *VE-Cadherin-CreER^{T2}*	no	Only one sex Cre+, no reported preference	has not been investigated but likely the same as *VE-Cadherin-CreER^{T2}*
	Cdh5(PAC)-CreER^{T2}	tamoxifen-inducible	has not been investigated but likely the same as *VE-Cadherin-CreER^{T2}*	no	Only one sex Cre+, no reported preference	has not been investigated but likely the same as *VE-Cadherin-CreER^{T2}*
MSC and perivascular Cre strains	*Prx1-Cre*	no	yes: although spine cells are not detected	none reported	Cre+ male mated to Cre− female	none reported
	Prx1-CreER-GFP	tamoxifen-inducible	yes: although spine cells are not detected	none reported	Cre+ male mated to Cre− female	none reported
	Nestin-Cre (Tronche strain)	no	no: central nervous system, some other cells in other organs detected	yes	Only one sex Cre+, no reported preference	none reported
	Nestin-Cre (Trumpp strain)	no	no: peripheral nerves, ECs, gastroinstestinal system, somites, some other cells in other organs detected	yes	Cre+ male mated to Cre− female	Cre recombinase activity reported to be weaker when inherited from Cre+ females
	LepR-Cre	no	no: some brain cells also detected	no	Only one sex Cre+, no reported preference	none reported

ECs, endothelial cells.

mice (Göthert et al., 2005). Compared to *Vav-Cre* mice, *HSC-SCL-Cre-ER^{T}* mice have relatively much lower Cre recombinase expression outside the hematopoietic system and no reported cases of germline Cre activation. However, when tamoxifen was administered prenatally, Cre recombinase activity was detected in ECs (Göthert et al., 2005). Some ECs were also targeted by *HSC-SCL-Cre-ER^{T}* when tamoxifen was administered to adult mice, but at a much lower frequency than the proportion of ECs targeted by *endothelial-SCL-Cre-ER^{T}* (Göthert et al., 2005). To date, our experience in using different HSC Cre strains has suggested that *HSC-SCL-Cre-ER^{T}* mice serve as the best model for efficiently and specifically targeting adult hematopoietic cells.

Different Cre Transgenic Mice Used to Detect HSC Niche Cells: Vascular and Perivascular Cells

EC Cre Transgenic Mice

Aside from the *Tie2-Cre* mice discussed above, there are a number of transgenic mice that can be used to detect and study functions of ECs (Table 2). There is a common theme that, similar to the EC targeting that occurs in many HSC "specific" strains, it is virtually impossible to avoid some Cre recombinase activity in hematopoietic cells when using EC promoters. This is likely due to the embryonic origin of both definitive hematopoiesis and endothelium, which arise from a shared precursor, the hemogenic endothelium (Hirschi, 2012). To try and minimize the contribution of Cre+ hematopoietic cells, wild-type BM can be transplanted into lethally irradiated EC-Cre strains; however, small proportions of endogenous HSCs survive lethal irradiation and can repopulate hematopoiesis in the transplanted mice, meaning that Cre+ hematopoietic cells have the capacity to contribute to the phenotype. Alternatively, Cre recombinase activity in hematopoietic cells can be reduced by inducible Cre recombinase activation in adult mice; however, this also is not without complications, as discussed below.

VE-Cadherin. There are at least four published strains of transgenic mice that target vascular endothelial (VE) cadherin (also known as cadherin 5, or Cdh5). Two were generated in

the Iruela-Arispe laboratory, the constitutively active VE-Cadherin-Cre (Alva et al., 2006) and the tamoxifen-inducible VE-Cadherin-CreER^{T2} (Monvoisin et al., 2006). Sun et al. (2005) generated a tetracycline-regulated transcriptional activator (tTA) under control of the VE-Cadherin promoter (VE-Cadherin:tTA). The fourth strain [Cdh5(PAC)-CreER^{T2}] was generated in the Adams laboratory by the insertion of a tamoxifen-inducible Cre into a large P1 artificial chromosome (PAC) clone of the Cdh5 gene (Sörensen et al., 2009). The constitutively active VE-Cadherin-Cre and the Cdh5(PAC)-CreER^{T2} strains have recently been used to show that endothelial-cell-derived Jagged1 is an essential regulator of HSCs (Poulos et al., 2013).

Cell specificity. There is a lack of published information on the specificity of the VE-Cadherin:tTA and Cdh5(PAC)-CreER^{T2} strains; however, the Iruela-Arispe laboratory has extensively examined Cre recombinase activity in numerous organs in embryonic and adult mice by lineage tracing, and it is likely that this specificity is retained in the other VE-Cadherin strains. In embryos, the majority of ECs in all organs showed Cre recombinase activity (Alva et al., 2006). However, significant proportions of targeted hematopoietic cells were also observed in the circulation, spleen, liver, thymus, and BM of E14.5 embryos. In adult BM, approximately 50% of the cells showed Cre recombinase activity in the constitutively active VE-Cadherin Cre strain (Alva et al., 2006). A separate study by the same group used flow cytometry (FACS) to demonstrate Cre recombinase activity in all hematopoietic cell lineages (Zovein et al., 2008). Some targeted cells were also detectable in the vessels of the thymus and in the white pulp of the spleen in the constitutively active VE-Cadherin Cre strain (Alva et al., 2006). It was unclear if these were ECs or hematopoietic cells.

In the tamoxifen-inducible model, hematopoietic cells were targeted when Cre was activated in embryos by injecting the mothers with tamoxifen at E9.5. These were shown to arise from the aortic-gonado-mesonephros (AGM) region (Zovein et al., 2008). Very few hematopoietic cells showed Cre recombinase activity when the Cre was activated postnatally (Monvoisin et al., 2006).

Note, however, that the authors observed an inverse correlation between the degree of excision and the age of the mice at which tamoxifen was administered (Monvoisin et al., 2006). It appears that inducing Cre recombinase in postnatal pups is preferred when aiming to reduce the extent of hematopoietic excision but that Cre recombinase activity in postnatally excised ECs may not be optimal, especially if using the inducible VE-Cadherin Cre strains to excise in older (6- to 8-week-old) mice. Similar issues with detection have also been observed with the *endothelial-SCL-Cre-ER^{T}* strain (Göthert et al., 2004); hence, it is possible that this also applies to other inducible EC strains.

MSCs and Perivascular Cre Strains

Prx1-Cre. Prx1 (also known as MHox) is a paired family homeobox gene expressed early during limb bud mesenchyme development (Martin et al., 1995). *Prx1-Cre* mice were recently used to achieve targeted deletion of chemokine (C-X-C motif) ligand 12 (CXCL12) in immature mesenchymal stem/progenitor cells. The resulting mobilization and depletion of HSCs in the BM demonstrated that these *Prx1-Cre*-expressing cells are an integral part of the HSC niche (Ding and Morrison, 2013; Greenbaum et al., 2013). Previous elegant studies by Sugiyama et al. used a CXCL12-GFP reporter mouse to describe the presence of CXCL12-abundant reticular (CAR) cells in the BM that had important roles in regulating HSC quiescence (Sugiyama et al., 2006). Greenbaum et al. (2013) recently demonstrated the existence of a small population of cells targeted by *Prx1-Cre* that coexpressed CXCL12-GFP and exhibited properties consistent with MSCs.

Cell specificity. Prx1-Cre transgenic mice display varied levels of germline deletion when the Cre transgene is transmitted through female heterozygous mice (Logan et al., 2002). Furthermore, the spine, which is of lumbar origin (Logan et al., 2002), is not targeted by Prx1-Cre, suggesting that Prx1-Cre cells do not give rise to all bone-forming cells in the body.

Nestin-Cre (Nes-Cre). Nestin is a type IV intermediate filament initially identified with a neuroepithelial-stem-cell-restrictive expression in the brain (Lendahl et al., 1990). However, nestin expression has been reported in a range of distinct brain- and tissue-specific progenitor cells (Wiese et al., 2004). Nestin expression by muscle or neural progenitors has been shown to be regulated by cell-specific regulatory elements in the first and second intronic region, respectively, of the rat nestin gene (Zimmerman et al., 1994). Using this knowledge, two different constitutively active *Nes-Cre* transgenic strains were generated using the 5.8 kb rat nestin promoter and intron 2 enhancer to drive Cre recombinase expression (Tronche et al., 1999; Trumpp et al., 1999). Both showed extensive recombination in the central nervous system (CNS) but also have many off-target effects as discussed below.

Recent studies from the Frenette laboratory used *Nes-GFP* reporter mice (Mignone et al., 2004), which were generated using similar constructs to the *Nes-Cre* strains, to identify perivascular nestin-expressing cells in the BM with in vitro MSC potential that contributed to the regulation of HSCs (Méndez-Ferrer et al., 2010). The Morrison lab used the *Nes-Cre* strain generated by Tronche et al. to show that deletion of CXCL12 from nestin-expressing cells had no impact on HSCs or hematopoiesis (Ding and Morrison, 2013). This is in contrast to the profound effects observed on HSCs when *Prx1-Cre* was used to delete CXCL12 in MSCs (Ding and Morrison, 2013; Greenbaum et al., 2013); hence, it is unlikely that *Nes-Cre*-targeted cells are the same cell as *Prx1-Cre*-targeted cells. It is possible that the *Nes-GFP* mice identify different cells to those reported by the *Nes-Cre* strains; however, this currently remains undetermined.

Cell specificity. The *Nes-Cre* transgenic strain developed by Tronche et al. has been reported to have Cre recombinase activity in tissues outside the brain including the kidney, retina, and heart (Martins et al., 2008; Tronche et al., 1999). Furthermore, the *Nes-Cre* transgenic mice developed by Trumpp et al. exhibited Cre recombinase activity in kidney, peripheral nerves, ECs, the gastrointestinal system, and somite tissues (Dubois et al., 2006). In addition, the Cre recombinase activity was weaker in the offspring when inherited thorough a female Cre+ parent (Dubois et al., 2006). Furthermore, despite studies from the Frenette lab showing MSC potential in vitro, very few cells giving rise to mesenchymal-derived lineages (chondrocytes, bone, and fat) were observed in the bones of the mice in vivo when the Tronche *Nes-Cre* mice were crossed to the R26R (LacZ) strain (Méndez-Ferrer et al., 2010); hence, further clarification of the nature of the BM-derived nestin-positive cells

Table 3. A Summary of Osteoblast-Cre Mice, Their Advantages, and Their Limitations

	Mouse Strain	Inducible/ Repressible	Specific to the Cell of Interest?	Germline Deletion?	Recommended Mating Strategy	Other Potential Issues Associated with This Strain
Osteoprogenitors (OPs)	*Osx1-GFP::Cre*	doxycycline-repressible	yes: but may not detect all Ob-lineage cells	no	Cre+ male mated to Cre– female	growth retardation (Davey et al., 2012) and malocclusion occur often in Cre+ mice including wild-types
	Osx-CreER^{T2}	tamoxifen-inducible	yes: but may not detect all Ob-lineage cells	no	Only one sex Cre+, no preference	none reported
Mature osteoblasts (Obs)	*Mouse Col2.3-Cre*	no	yes	no	Only one sex Cre+, no reported preference	none reported
	Rat Col2.3-Cre	no	no: some expression in articular cartilage, occasional activity in growth plate chondrocytes	yes	Cre+ male mated to Cre– female	none reported
	Rat Col3.6-Cre	no	no: also present in tendon, fascia fibroblasts; some expression in articular cartilage, occasional activity in growth plate chondrocytes	yes	Cre+ male mated to Cre– female	none reported
Osteocytes (Ocys)	*Dmp1-Cre (8 kb)*	no	no: present in some brain cells	no	Only one sex Cre+, no reported preference	none reported
	Dmp1-Cre (9.6 kb)	no	no: present in Obs, muscle, intestines, and brain	no	Only one sex Cre+, no reported preference	none reported

Ob, osteoblast.

is required. More importantly, both of the *Nes-Cre* lines display germline activation (Dworkin et al., 2009). Inducible *Nes-Cre* transgenic strains generated using similar constructs are available (Balordi and Fishell, 2007; Chen et al., 2009), and hence may overcome this latter issue.

Leptin Receptor-Cre (LepR-Cre). Leptin is an adipocyte secreted circulating hormone that acts through the leptin receptor (LepR) to regulate energy metabolism in the body. Recently, using the stem cell factor (*Scf*)^{gfp} knockin mice, Ding et al. (2012) determined that *Scf*^{gfp}-expressing BM perivascular stromal cells expressed LepR. Furthermore, *LepR-Cre*-based deletion of SCF negatively affected HSC maintenance in the BM. These cells were identified to be distinct from the *Nes-Cre* targeted perivascular BM stromal cells (Ding et al., 2012). However, a recent study using *LepR-Cre*-tdTomato reporter mice crossed to *Nes*^{gfp} mice showed that the majority of LepR-targeted cells express nestin (Pinho et al., 2013). Further studies are warranted to conclusively determine the nature of these cells.

Cell specificity. The *LepR-Cre* mice have been reported to be brain specific (Plum et al., 2007; Scott et al., 2009); however, the lineage tracing studies have not been extensive to date and have omitted studies in hematopoietic organs. Given the phenotype observed by Ding et al. (2012), a more comprehensive lineage tracing study is warranted to identify the BM-containing LepR-positive cells.

Ob-Lineage Cre Mice

The bone cavity and BM in which hematopoiesis occurs contain a range of Ob-lineage cells, differentiating from immature mesenchymal/skeletal stem cells (discussed above) to osteoprogenitors (OPs) to mature bone-forming Obs and ultimately osteocytes (Ocys) embedded in the bone matrix (Askmyr et al., 2009). The strains that have been most commonly used to show distinct roles for differently staged Ob-lineage cells in hematopoiesis are discussed below (Table 3), and others have also been overviewed by Elefteriou and Yang (2011).

Osx1-Cre (OPs). Osterix (Osx) is a zinc-finger-containing transcription factor, essential for Ob differentiation and bone formation (Nakashima et al., 2002). Osx expression is switched on at the OP stage of osteogenic differentiation, downstream of the transcription factor Runx2. *Osx1-GFP::Cre* transgenic mice were generated by pronuclear injection of a BAC containing the mouse *Sp7* gene (encoding *Osx*) targeted at exon 1 (Rodda and McMahon, 2006). In these mice a tetracycline (Tet)-off controlled GFP::Cre fusion protein is under the transcriptional control of the *Sp7/Osx1* promoter, allowing repression of Cre recombinase by administration of doxycycline (Rodda and

McMahon, 2006; Walkley et al., 2008). Maes et al. (2007, 2010) separately generated, by BAC methods, tamoxifen-inducible *Osx-CreER*^{T2} mice in which CreER^{T2} is under the control of the Osx gene promoter.

Studies using the *Osx1-GFP::Cre* mice have been used to show that deletion of the heterotrimeric G protein α subunit (Gsα) or components of the HIF signaling pathway in *Osx1*+ cells impaired B cell lymphopoiesis (Wu et al., 2008) or erythropoiesis (Rankin et al., 2012), respectively. Furthermore, deletion of CXCL12 in *Osx1-GFP::Cre* mice resulted in hematopoietic progenitor cell (HPC) mobilization and a loss of B lymphoid progenitors but did not affect HSCs (Greenbaum et al., 2013).

Cell specificity. Lineage tracing studies in embryos, neonates, and adult mice using either strains have shown that *Osx1-Cre* targets Ob-lineage cells in the perichondrium/periosteum, within the trabecular and cortical bone regions and in the skull bones (Maes et al., 2010; Rankin et al., 2012; Rodda and McMahon, 2006). Stromal cells inside the developing bone (considered to be immature OPs) also expressed Cre recombinase, as did the mature Ocys. There was also some Cre recombinase activity detected in the lower zone of columnar chondrocytes and in pre-hypertrophic chondrocytes, consistent with known expression of Osx in these cell types (Maes et al., 2010; Rankin et al., 2012; Rodda and McMahon, 2006). Interestingly, a subset of Osx+ cells in either strain was noted to be perivascular in embryonic and adult mice, and these are considered to be OPs migrating to the bone during development and repair after injury (Maes et al., 2010).

Col1α1-Cre (mature Obs). Two different mouse strains of the *Col2.3-Cre* mice have been made using either the mouse Col2.3 promoter (Dacquin et al., 2002) or the rat Col2.3 promoter (Liu et al., 2004) to drive Cre recombinase. In each of these strains, the Col2.3 promoter efficiently targeted all mature Obs in the body and virtually no Cre recombinase activity was observed in other tissues (Dacquin et al., 2002; Liu et al., 2004). Transgenic mice generated using the rat 3.6 kb Col1α1 promoter exhibit Cre recombinase expression in early Obs, perichondrium, and suture mesenchyme (Liu et al., 2004). In addition, both the 2.3 kb *Col1α1-Cre* and 3.6kb *Col1α1-Cre* mice generated using the rat promoters displayed Cre recombinase activity in articular cartilage, with occasional activity detected in growth plate chondrocytes (Liu et al., 2004).

The mouse promoter-derived *Col2.3-Cre* mice have been used to demonstrate important roles for osteoblastic connexin 43 in regulating HSCs (Gonzalez-Nieto et al., 2012) and to show that mature Obs do not regulate HSCs via N-cadherin (Bromberg et al., 2012; Gonzalez-Nieto et al., 2012). Ding et al. used the rat Col2.3-Cre mice to show that deletion of CXCL12 (Ding and Morrison, 2013) or SCF (Ding et al., 2012) in Ob-lineage cells did not impair HSC function. BM from the *Col2.3-Cre-Cxcl12*$^{fl/fl}$ mice was, however, reported to have impaired lymphopoiesis when transplanted into wild-type recipient mice (Ding and Morrison, 2013). Furthermore, *Col2.3:GFP* mice, which were generated using the same rat reporter as the *Col2.3-Cre* mice (Dacic et al., 2001), were used to show that HSCs localize adjacent to GFP+ Obs at early time points after transplantation (Lo Celso et al., 2009).

Cell specificity. Another constitutively active Cre strain generated using the rat 2.3 kb Col1α1 promoter targeted rare CD45+ fibrocyte-like cells in BM (Ohishi et al., 2012); hence, this may also apply to the other rat 2.3 kb *Col1α1-Cre* mice. Furthermore, the *Col1α1-Cre* transgenic mice employing rat promoters have reported cases of germline activation transmitted at a higher incidence through females (Cochrane et al., 2007; Liu et al., 2004; Scheller et al., 2010). It has also been reported in 3.6 kb *Col1α1-Cre-p53*$^{fl/fl}$ mice that approximately 20% of mice develop lymphoma (Lengner et al., 2006). While it is possible that changes in the microenvironment could contribute to the lymphoma, lineage tracing studies are required to show that hematopoietic cells are not targeted by 3.6 kb *Col1α1-Cre*.

Dmp1-Cre (Ocys). Dentin matrix acidic phosphoprotein (Dmp1) is an extracellular matrix protein expressed highly in mineralized tissue such as that by Ocys in bone and by odontoblasts in teeth (Feng et al., 2003). Transgenic mice generated using the 8 kb promoter region of Dmp1 to drive GFP expression found its expression restricted to osteocytic cells of the bone lineage, odontoblast, and a few brain cells (Kalajzic et al., 2004). Furthermore, Lu et al. (2007) generated *Dmp1-Cre* transgenic mice by placing Cre recombinase under the control of a 9.6 kb Dmp1 promoter. These *Dmp1-Cre* mice displayed Cre recombinase activity in Ocys, odontoblasts, and a few Obs. They were recently used to demonstrate defective myelopoiesis in mice bearing Ocy-specific deletion of Gsα (Fulzele et al., 2013).

Cell specificity. The 9.6 kb *Dmp1-Cre* strain has been reported to have off-target activity in Obs, muscle, intestine, and brain (Xiao et al., 2011; Xiong et al., 2011). Recently generated 8 kb *Dmp1-Cre* have shown increased specificity to Ocys with no reported Cre recombinase activity in Obs (Bivi et al., 2012) and may provide better insight into the contribution of Ocys to the regulation of hematopoiesis.

Reporter Mice Used to Detect or Ablate HSCs or HSC Niche Cells

The use of lineage tracing has been a significant advance in the application of genetically engineered mouse models to understand HSCs and the BM microenvironment (Blanpain, 2013; Blanpain and Simons, 2013; Fuchs and Horsley, 2011). The permanent identification of the cells generated from the Cre of interest allows identification of these cells and can also provide important information regarding the specificity of the Cre line for the proposed target population. These reporters serve several purposes and can be applied for multiple reasons. First, they permit the identification and isolation of cells in which Cre has been activated and also permit analysis of the specificity of a given Cre for a lineage. A major caveat is that the activated reporters mark all progeny of cells in which Cre has been expressed. For example, if Cre is expressed in an HSC, the reporter's expression will be detected in all hematopoietic cells that come from that HSC. The expression of Cre developmentally may be different from that in the adult and, as mentioned above for EC Cre strains, constitutive Cre lines can lead to the presence of labeled cells where they would not have been expected as a result of these developmental differences.

The use of lineage reporters allows visualization of the cells that are contributing to a phenotype and their ready isolation, in most cases by flow cytometry. An alternative use of these lines is to allow pulse chase analysis of the life span of a population

of cells (Park et al., 2012). This is particularly useful when inducible Cre lines exist for the cell populations of interest. The rate at which a population loses the induced mark can be used to determine the rate of turnover of that population in vivo.

Until recently, most reporter systems were a binary model of off and on with the expression of a fluorophore, most commonly GFP or derivatives. New, more sophisticated models have been developed that allow for clonal analysis and color substitutions.

Rosa26 Strains

One of the most widely used reporter models incorporates a loxP flanked stop cassette into the Rosa26 (R26) locus (Soriano, 1999). The R26 strain shows ubiquitous expression in embryos and adult tissues (Soriano et al., 1991; Zambrowicz et al., 1997). Furthermore, homozygous R26 mice are viable, making it possible to breed two different strains of R26 mice (e.g. a deleter and a reporter strain) together for experimental purposes. When used in reporter strains, after Cre exposure the stop cassette is removed and expression of the reporter construct is initiated. These models have been used with a range of reporters including LacZ, GFP, and eYFP (Mao et al., 1999; Srinivas et al., 2001). The R26 strains have been widely used for many studies and there are many variations on this approach incorporating alternative loci, more highly expressed promoters, or multiple fluorophores, which allow tracing of cells exposed to Cre (e.g. membrane-targeted GFP, mG) and an alternate label (membrane-targeted tandem dimer tomato, mT) for unexposed cells (Madisen et al., 2010; Muzumdar et al., 2007). At present the Jackson laboratory repository offers hundreds of different variations of R26 fluorescently labeled or LacZ-expressing strains. It has been observed, however, that there can be variegated expression of the Rosa26 locus, the impact of which needs to be assessed in each application (Hameyer et al., 2007).

Multicolored Reporter Mice

A variation on the binary reporter systems has been the development of multicolored reporters that rearrange following Cre activation and mark a clonal population with one of four or more fluorophores. These mice include the ''Brainbow'' mice (restricted to brain studies due to the nature of the reporter), which generates at least 90 colors (Livet et al., 2007), and the ''Confetti'' mice, which form four different fluorophores that can be detected by FACS and in tissue sections (Snippert et al., 2010).

The multicolored approaches permitted by the Brainbow and Confetti reporters have been revelatory in understanding the clonal contribution of stem cells, especially when coupled with inducible Cre models where a temporal exposure to ligand results in Cre activity for a short period of time. This experimental approach leads to the generation of clonally identifiable stem cell populations whose behavior and contribution to organ homeostasis can be measured. The Confetti reporter mice have recently been used to identify hemospheres consisting of ECs, mesenchymal cells, and hematopoietic cells that support HSC clonal expansion (Wang et al., 2013). When combined with advanced imaging and analysis, these powerful reporter strains can reveal new biology about the in vivo relationship of distinct cell populations.

Conditional Ablation of Cell Lineages: Thymidine-Kinase- or Diptheria-Toxin-Expressing Mice

The two previously discussed reporter mouse approaches utilize Cre to mark cells with one or more tracers to allow further analysis. An alternative is to specifically remove a cell population and then assess the consequences of this on homeostasis of the organ. This is made possible by generating mice that express either a truncated form of the herpes simplex virus-thymidine kinase (HSV-tk) gene (Visnjic et al., 2001) or the diptheria toxin receptor (DTR), under the control of the promoter gene of the cell type of interest (Brockschnieder et al., 2004; Saito et al., 2001). Injection of the resulting mice with either ganciclovir or diptheria toxin, respectively, results in selective ablation of the targeted cell.

The HSV-tk enzyme is not toxic to the mammalian system. However, HSV-tk phosphorylates specific nucleoside analogs (such as ganciclovir or acyclovir) to the nucleoside monophosphate. This, in turn, is phosphorylated into the nucleoside triphosphate and incorporated into DNA, inhibiting DNA synthesis and hence resulting in cell death in the cell type expressing HSV-tk, but leaving the other cell types unharmed (Heyman et al., 1989). The tk gene causes sterility problems in transgenic males, but this can be overcome using a truncated, functional form of the enzyme (Δtk). Visnjic et al. (2001) achieved Ob-specific ablation using rat Col2.3Δtk mice injected with ganciclovir, demonstrating that ablation of Obs resulted in numerous hematopoietic defects, including mobilization of HSCs (Visnjic et al., 2004).

The DTR is not naturally expressed by mice (Cha et al., 2003), allowing for cell-specific loss of viability by engineering mice to express DTR in the desired cell of interest. Cell deletion can be achieved by injecting the mice with diptheria toxin. Méndez-Ferrer et al. (2010) used this approach to deplete nestin+ cells in mice, showing that HSCs were mobilized from the BM but that other hematopoietic cell types were not affected. Furthermore, Asada et al. (2013) used Dmp1-DTR mice to demonstrate that ablation of Ocys resulted in failure of mobilization of HSCs and progenitor cells in response to G-CSF.

Additional Methods Used to Detect HSCs: Label Retention

In addition to the use of reporter mice for HSC studies, other methods can be used to label HSCs based on their quiescence. HSCs, as is the case with many other somatic stem cells, have long been known to be quiescent, especially when compared to the highly proliferative HPCs they give rise to. Genetic or chemical labeling of quiescent cells based on initial uniform label (pulse), followed by label dilution during cell division events (chase), has therefore proven useful to identify a subpopulation of HSCs that are particularly quiescent (or dormant), have the highest reconstitution potential, and respond to specific injuries by proliferating and giving rise to differentiating progeny. Pioneering studies by Bradford et al. (1997) used continuous oral bromodeoxyuridine (BrdU) administration (which incorporates into the DNA of dividing cells) to demonstrate that the most primitive HSCs cycle slowly, with an average turnover time of 30 days, and to show that there is a hierarchy of HSCs based on quiescent properties. An additional study by Wilson et al. (2008) identified label-retaining HSCs (LRCs) by two methods in parallel: BrdU administration and doxycycline-regulated expression of the fusion protein histone 2B-GFP (H2B-GFP), which had been generated to identify label-retaining cells in the epidermis (Tumbar et al., 2004). While BrdU detection

requires cell fixation and permeabilization, retention of H2B-GFP allows FACS-based purification, transcriptional profiling, and functional testing of LRCs.

Caution must be taken with the administration of BrdU. If injected into the animal, BrdU will only be incorporated into the DNA of cells that are dividing at the time of injection. Hence the cells that do not contain BrdU after a short period of administration of BrdU are not necessarily HSCs. The best way of administering BrdU to ensure HSC labeling is by adding it to the drinking water of the mice in bottles that are protected from light for extended periods of time.

There are also experimental limitations of BrdU studies. There are concerns that the BrdU method may not sufficiently detect all HSCs (Kiel et al., 2007). Furthermore, BrdU has been shown to lead to activation of dormant HSCs and therefore to inherently alter the physiology of the tissue (Wilson et al., 2008). Regardless of these issues, label retention studies combined with immunofluorescence techniques permitted the identification of dormant HSCs and suggested that different types of HSCs reside in specific niches that may be composed of unique combinations of cell types, most likely located in specific BM areas.

Emerging Technologies to Visualize HSC Interactions with Their Niches

Confocal Laser Scanning Microscopy

Several groups have used a variety of approaches to genetically or chemically label HSPCs (recently reviewed in Progatzky et al., 2013), transplant them, and study their homing within the BM space of recipient mice by means of histological analysis of tissue sections. Functional, engrafting HSCs are only observed in myeloablated recipients; however, the irradiation procedures alter important cell types that contribute to the niches, including ECs (Hooper et al., 2009) and Obs (Dominici et al., 2009), hence presenting a limitation to the knowledge obtained using transplanted HSCs. The development of the SLAM markers signature, allowing identification of HSCs based on staining with a limited number of antibodies, led to the first analysis of HSC localization in nonperturbed BM sections using confocal laser scanning microscopy (CLSM) (Kiel et al., 2005), and histological studies of BM sections are currently very popular. These localization studies are, however, not without their limitations: the complexity of the HSC niche is not fully captured by 2D sections. This can be partly overcome by performing serial sectioning, which however remains challenging and, due to restrictions in section thickness and width, might not be able to capture all interactions of the HSC with surrounding microenvironment cells. Furthermore, CLSM studies are not performed in real time with living cells, and dynamic interactions cannot be captured by single time-point analysis.

Intravital Microscopy

Intravital microscopy solved all these initial limitations and has been used to observe the behavior of HSPCs in real time in multiple bone areas. Epifluorescence microscopy was used to pioneer imaging in the BM contained within the mouse skull frontal bones (Mazo et al., 1998), and higher resolution images were obtained using confocal (Sipkins et al., 2005) and combined confocal and two-photon microscopy (Lo Celso et al., 2009; Sipkins et al., 2005). In particular, the combination of confocal and two-photon microscopy allows expanding the palette of analyzable labels to span from bone collagen second harmonic generation signal to near-infrared Quantum dots such as Qdot800, permitting the monitoring of multiple components of the HSC niche simultaneously (Lo Celso et al., 2011). HSCs localized in femur and tibia BM have been observed by intravital microscopy following insertion of an endoscopic probe from the knee (Lewandowski et al., 2010) and exposure and surgical thinning of the compact bone (Köhler et al., 2009), respectively. Both of these approaches are effective; however, they are highly invasive and therefore allow only a single imaging session to be performed. Imaging has also been successfully performed to detect the homing of HSCs in halved femurs at early time points posttransplantation; however, this required euthanasia of the animal prior to ex vivo imaging of the sample (Xie et al., 2009). Instead, calvarium imaging is minimally invasive, and even though it is restricted to observing a relatively small proportion of exclusively narrow BM cavities, it is the only imaging modality to date that permits the investigator to monitor HSCs for longer periods of time over multiple imaging sessions (Lo Celso et al., 2009).

Since HSC-specific reporter transgenic lines are not currently available, all intravital imaging approaches are based on transplantation models, requiring myeloablative conditioning of the recipients. These studies have therefore been providing insights on HSC-niche interactions during early engraftment, at a time in which both HSCs and the BM microenvironment are exposed to very high levels of stress. The use of engraftment-permissive lines such as W/W^{v} mice (Migliaccio et al., 1999) avoids the need for harsh conditioning methods, but still has the limitation that the microenvironment observed is not wild-type. The development of new reporter strategies is greatly needed to obtain imaging of HSCs in situ, in physiological conditions.

Laser Scanning Cytometry

Another, more recently developed technology is becoming popular in studies for imaging HSCs: laser scanning cytometry (termed LaSC here so as not to confuse with leukemia stem cells). Unlike intravital microscopy studies, LaSC does not require transplantation of HSCs into perturbed microenvironments, permitting in situ analysis. While limited in that they cannot perform live-cell imaging, the noninvasive studies made possible by both LaSC and CLSM provide complementary and alternative methods for studying HSC-niche interactions.

The LaSC is an automated, cellular analyzer that combines the imaging resolution of CLSM with the quantitative capabilities of FACS (Henriksen et al., 2011). The union of these technologies permits the LaSC to measure multiple fluorescently labeled cells on adherent specimens including tissue samples/biopsies, cytology smears, and cultured cells (Tárnok and Gerstner, 2002). The advantage of preserving tissue architecture is that it allows the acquisition of morphological and subcellular components of individual cells in their natural microenvironment. The LaSC additionally records the precise spatial coordinates of a molecule or cell within the section and permits the construction of detailed tissue maps. This feature also makes it possible for researchers to study molecular and cellular events and interactions in situ, a platform that has not been available before (Harnett, 2007; Nombela-Arrieta et al., 2013; Oswald et al., 2004; Zhao et al., 2010).

The LaSC also differs from FACS in that samples are not discarded postacquisition and can therefore be restained and reanalyzed, a feature that may be invaluable for research and clinical samples that have very little cells, in particular samples obtained from fine-needle aspiration biopsies. Several clinical studies have reported the successful use of the LaSC for the immunophenotyping of hematological specimens (Clatch et al., 1996, 1998), for measuring DNA content (Clatch and Foreman, 1998; Wojcik et al., 2001; Zabaglo et al., 2003), and for tissue histology (Gerstner et al., 2004). Results from these studies were comparable to conventional immunocytochemistry analyses, with advantages for certain applications including the ability to correlate individual cell morphology to fluorescence data, reduced specimen and reagent use, exclusion of pathologist interpretations, simplified methodologies, and high reproducibility.

LaSC technology is distinct from CLSM in that it collimates the laser beam (i.e. makes the beam as parallel as possible) to allow all the emitted light to be quantified from the entire depth of the cell. This increases both the depth of field (typically 20 to 30 μm) and the area that can be scanned in focus, thereby allowing automated analysis possible on relatively large sections of tissue. In contrast, CLSM analyses light emitted near the focal plane to provide excellent spatial resolution in a small optical area, but this also permits serial optical sectioning of samples and their reconstruction into 3D images. Due to these differences, LaSC can be elegantly complemented by CLSM, as recently exemplified by a study by Nombela-Arrieta et al. (2013). This study adapted the use of the LaSC to quantify the distribution of three defined populations of hematopoietic stem and progenitor cells (HSPCs) within whole BM tissue sections. After showing that HSPCs preferentially localized in endosteal regions of bone, closer to blood vessels, they further confirmed their findings with detailed 3D reconstructions of BM microvasculature using CLSM.

Whole-Mount Imaging and Computational Modeling Methods

The employment of new quantitative imaging technologies will help us to define key factors involved in the regulation of HSCs in their microenvironment. In support of this, recent developments using whole-mount confocal immunofluorescence imaging techniques combined with computational modeling have been optimized to obtain the first 3D reconstructions of the BM within the bone cavity in its entirety (Kunisaki et al., 2013). This revealed that small arterioles ensheathed by pericytes that coexpressed nestin, α-smooth muscle cell actin, and the glial marker NG2 have critical roles in regulating quiescent HSCs (Kunisaki et al., 2013).

Concluding Remarks

In conclusion, a range of valuable genetically modified mouse strains, combined with reporters and advanced imaging technologies, have begun to help unravel important regulatory mechanisms of HSC niches. While these have brought great advances to our understanding of how HSCs are regulated by their microenvironments, there is a clear need to improve our understanding of HSC-niche interactions and to convert these data into biologically and clinically informative results. Studies using human biopsies are likely to become more commonplace for HSC-niche interaction studies with the increasing use of technologies such as laser scanning cytometry (Henriksen et al., 2011) and laser confocal microscopy studies (Takaku et al., 2010). While we still have far to go, HSC niche research is increasingly evolving and highly exciting.

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